Fish Oils-Volume 1 #104 12/5/73

FISH OILS VOL I #104

M23

VOLUME 1

GRAS MONOGRAPH SERIES

FISH OILS

prepared for

THE FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH, EDUCATION
AND WELFARE

DECEMBER 5, 1973

This publication was prepared under Contract Number FDA 72-100
with the Public Health Service, Food and Drug Administration,
Department of Health, Education, and Welfare

prepared by

Tracor Jitco, Inc.

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SUMMARY

Description and Specifications

For present purposes fish oil may be defined as the oil expressed primarily from menhaden, and secondarily from herring or tuna (839). Outside the United States, herring is currently the primary source (828). The California sardine (pilchard) was finally overfished in 1951 (839), the Peruvian anchovy in 1972, probably (386). Whale oil can no longer be used legally in the United States.

Fish oils are especially rich in long chain polyunsaturated fatty acids (PUFA), with more than C_{18} and with four or more double bonds (049,305,836). About 2% of their contents have essential fatty acid (EFA) activity (730). They also contain small amounts of volatile short chain acids (176), phospholipids, ether-linked compounds, hydrocarbons, wax esters and sterols, such as cholesterol and "vitamin" D (558,836,501,496).

The purpose of hydrogenation is to elaborate a stable, palatable product of suitable plasticity (028). Hydrogenation is a variable process that saturates some but not all of the double bonds; the aim is to partly-saturate polyene chains but not monoene (028). Therefore, hydrogenated fish oils are never more than partly hydrogenated. The proportion of double bonds left unsaturated is reflected by the iodine number. But the fatty acid (FA) composition is altered, in most cases not very predictably (028,324,502,635,864).

Manufacturers patent their particular hydrogenation procedures to protect products with special handling characteristics (621,624,625,649). Thus, hydrogenated fish oils include a wide range of chemically different products that vary according to source and subsequent treatment (864).

The two important food uses of hydrogenated fish oils are in margarines and shortenings. Currently in the United States, these oils are used only in non-food industrial products for example, in paints and lubricants. This situation may change, however (828,836). (See below, Consumer Exposure.)

At present there is no standard of identity for hydrogenated fish oils that may be used in the United States' food supply (188,053,898,899,828). The nearest approach is the FAO/WHO Codex Alimentarius Recommended International General Standard for Edible Fats and Oils Not Covered by Individual Codex Standards (181). This sets maxima for acid and peroxide values, stabilizers, and other additive contents, and for contaminants such as lead, copper, arsenic, iron, and soap.

Part of the trouble has been a lack of usable routine analyses for the major components of hydrogenated fish oils. Very recently, development of sophisticated fractionation procedures and variants of gas-liquid chromatography have begun to overcome this lack (560,561,564, 718,380,028,835). Also fish oils have been regarded as

auxiliary sources of fats that are major nutrients, rather than as separately identifiable additives. From this viewpoint, hydrogenation introduces an uncontrolled and unwanted variable into research studies.

Consequently, the literature contains a great deal of information on fish oils but relatively little on hydrogenated fish oils (901,622,502). This monograph necessarily draws on the former for supportive data.

Acute Toxicity

Only one series of studies on the acute toxicity of fish oils was found. Between 1954 and 1962, Matsuo, in Japan, searched the degradation products of cuttlefish oil and found a cyclic monomer that was acutely toxic to rats and rabbits. He concluded that this monomer was an end product of autoxidation and peroxidation of many edible oils. The starting material was not hydrogenated (585,586, 597,598,599).

Short Term Studies

No studies on hydrogenated fish oils in man were found. No animal studies were found on hydrogenated fish oils used to supplement normal diets. Most studies that were identified are on fresh fish oils, on fractions of fish oils, on FA that occur in fish and other oils, or on derivatives and degradation products where the starting material happened to be a fish oil. In some cases defatted or semipurified diets were supplemented with

large amounts of the experimental substances, or of a hydrogenated fish oil.

Reports summarized in this section of the monograph include those of Haven (345), Dam (204), Blaxter et al. (111,112,113), Dam (204), Ershoff (251), Andrews et al. (058), Gershoff and Norkin (286), Rasheed et al. (739), Carpenter et al. (163), Roubal and Tappel (760), Stansby (832), Njaa et al. (668), and Grover (303).

From these studies emerged three major conclusions, applying to hydrogenated fish oils and all other dietary fats and oils:

1. Toxic effects reflect mainly deficiencies resulting from imbalances in the total experimental diets. In many studies the deficiency was of EFA activity (204,111,007,836).

2. Increased intakes of PUFA increase the requirements for vitamin E (112), largely but not wholly for its antioxidant properties (112,739). Calculations by Harris and Embree (333) suggested that in 1960 the population of the United States (consuming no fish oil) received only marginally sufficient vitamin E. However, this has been denied recently by the National Academy of Sciences (657). According to Hannewijk (324), carefully refined and hydrogenated fish oils contain adequate antioxidant activity. Many of the studies that were reviewed, however, reported toxic symptoms of vitamin E deficiency (202,286).

3. If oil putrefies by autoxidation, peroxides form, and if these are not destroyed in the intestine, results can be toxic (058,739). For example, free radicals arise that can damage proteins and amino acids (204,760). The more highly unsaturated the PUFA, the more readily the oil autoxidizes (832). According to Stansby (832) these are "all only potential effects" that can be "controlled by the use to which the oil is put."

Theoretically, peroxides can give rise to epoxides, which can be carcinogenic (303). But no studies were found to suggest that this might occur in vitro or in vivo with hydrogenated fish oils. On the contrary, Haven (345) reported that induced cancer in rats grew more slowly when the dietary fat was an extremely hydrogenated fish oil than when it was a highly unsaturated vegetable oil.

Long Term Studies

No relevant long-term studies were found in the literature. The controversial question of the general relationships of saturated/unsaturated fats, cholesterol, and atherogenesis was considered to be outside the scope of a monograph restricted to hydrogenated fish oils.

Special Studies

Evidence from the U.S. National Marine Fisheries Service (848,849) is reviewed to the effect that in some areas sea fish contain unacceptable levels of DDT and related pesticides, attributable to agricultural run-off. Stout and her colleagues (849) have established that these residues are substantially eliminated by the normal refining

and hydrogenation of fish oils. However, problems with the detection and elimination of PCB's remain (094).

Breakdown and Metabolism

Oxidation results in "off" flavors, and hydrogenation generates "hardening" flavors that are removed by further refining. The latter arise from small amounts of free or bound aldehydes (174), especially 6-*trans*-nonenal, which can sometimes be detected at a dilution of 0.3 ppb (620, 458,324). Off or hardening flavors can return during storage, and such oils become unacceptable to the taste before they become toxic (828). Stansby (828) notes that hydrogenated menhaden oil is no exception but, being only moderately unsaturated, should cause no special problems.

No reports were found to suggest that the digestion, absorption, or distribution of hydrogenated fish oils might differ from those of other dietary fats. However, a paper by McCay and Paul (607) suggests that hydrogenation may diminish absorption of any oils and thus increase the amounts excreted in the feces. In general, the FA distribution in the tissues tends to reflect that in the diet, taking into account that certain FA are readily metabolized or liable to inhibit the metabolism of other FA. This was shown in rats fed hydrogenated herring oil (090), in turkeys fed anchovy oil (660), and in Japanese fishermen with high intakes of fish oils (388).

Although EFA activity of fish oils (unhydrogenated) is equivalent to that of corn oil, a common source of EFA (730), much of this activity seems to be lost on hydrogenation (007); the reasons are not clear, however, and further research has been recommended (007). Losses of vitamins, including vitamin E, through hydrogenation also appear to require further study (913); van der Steur (913) has pointed to risks if they are over-replaced.

The literature contains many reports of metabolic effects of fish oils, and of the presence or lack of FA that are found in fish oils--see especially a cholesterol-lowering effect reviewed by Stansby (836)--but there is no estimate of the relevance of these reports to hydrogenated fish oils. This monograph does not survey the vast field of knowledge of FA metabolism and its interactions with other nutrients and metabolic pathways.

Drug Interactions — No reports have been found.

Consumer Exposure — Until the disappearance of the California sardine in 1951, large amounts of hydrogenated fish oils were made into margarine (839). Then the use of fish oils ceased; in 1955, when the present FDA standard of identity for oleomargarine (067) was set up, nobody asked for fish oils to be included (839). Today, therefore, hydrogenated fish oils cannot legally be included in margarine in the United States, although they are used extensively abroad (839).

CHEMICAL INFORMATION

I. Nomenclature

A. Common Names: Fish Oil (Hydrogenated); Hydrogenated Fish Oil.

B. Chemical Names: None for the entire product.

C. Trade Names· Either the common names are used or the species of fish that yielded the oil in the sample referred to. Examples are: Menhaden Oil, Herring Oil, Tuna Oil, etc. qualified by the adjective "hydrogenated," or "partly hydrogenated", or even "partially (sic) hydrogenated".

Currently, the menhaden is the predominant species of fish used as a source of fish oil (hydrogenated) in the United States. Four principal species of menhaden have been identified in the areas of the U.S. fisheries, two in the Atlantic Ocean and two in the Gulf of Mexico. The two commonest are Brevoortia tyrannus in the Atlantic, and B. patronus in the Gulf of Mexico (839). No references, however, were found in which a more precise term was used than "menhaden oil".

D. Chemical Abstracts Services Unique Registry Number: MX 8016 14 6.

NOTE: Relatively few reports on hydrogenated or partly hydrogenated fish oils were found, compared to the number on unhydrogenated fish oils. Some of the latter reports are therefore included as supporting data.

II. Empirical Formula

The composition of fish oil varies greatly according to its source. Stansby (836) points to "vast differences in composition of oils from different species of fish or even from oils of the same species of fish taken from different geographical areas," and also from different parts of the same fish. Major species of fish used as oil sources have tended to vanish, because of overfishing. One example was the Hokkaido herring. California sardine oil was last available in 1951 (828), and 1972 may have seen the end of the Peruvian anchovy as a major source of oil (386). Currently, the menhaden is the major source of fish oil in the United States, but production declined between 1960 and 1967 (839). Elsewhere the herring is the major oil source. Tuna is a less important source.

Fish liver oils, usually sold as carriers of vitamins A and D in amounts that are now categorized as "drugs" or "diet supplements", are outside the scope of this monograph. Whales are now "endangered species" whose oils cannot legally be marketed in the United States.

Table 1 shows the fatty acid (FA) compositions of menhaden and herring oils according to Altman and Dittmer (049). Compositions listed by Gruger (305) and by Stansby (836) differ considerably, but all authors have emphasized variability. Some of these fatty acids are described more fully in Tables 2 and 3 (from Altman and Dittmer 049). Fish oils are especially rich in long chain polyunsaturated fatty acids (PUFA).

The essential fatty acid (EFA) properties of the three fish oils most important to the fish oil production of the United States (menhaden, herring, and tuna oils) were investigated by Privett et al. (730),

Table 1

Typical Fatty-Acid Composition of Two Fish Oils*

FASEB Serial No.	Common name of fatty acid	Concentration g/100 g total fatty acids
	Menhaden Oil	
(74)	Myristic acid	5.9
(75)	Palmitic acid	10.3
(76)	Stearic acid	0.6
(77)	Arachidic acid	0.6
(78)	Behenic acid	0.8
(79)	Palmitoleic acid	15.5
(80)	Linolenic acid	29.6
(81)	Eicosapolyenoic acid	19.0
(82)	Docosapolyenoic acid	11.7
	Herring Oil	
(67)	Myristic acid	7.3
(68)	Palmitic acid	13.0
(69)	Stearic acid	Trace
(70)	Palmitoleic acid	4.9
(71)	Linolenic acid	20.7
(72)	Eicosapolyenoic acid	30.1
(73)	Docosapolyenoic acid	23.2

* Adapted from R.P. Geyer (Table 43) in Altman and Dittmer (049).

Table 2

Fatty Acids Found in Fish Oils*

FASEB Serial No.	Identity	Common name	Empirical formula	Sources
	Saturated			
(14)	Tetradecanoic	Myristic	$C_{14}H_{28}O_2$	see Table 1
(16)	Hexadecanoic	Palmitic	$C_{16}H_{32}O_2$	see Table 1
(18)	Octadecanoic	Stearic	$C_{18}H_{36}O_2$	see Table 1
(20)	Eicosanoic	Arachidic	$C_{20}H_{40}O_2$	see Table 1
(22)	Docosanoic	Behenic	$C_{22}H_{44}O_2$	see Table 1
	Unsaturated - monoethenoic			
(95)	cis-9-Hexadecenoic	Palmitoleic	$C_{16}H_{30}O_2$	see Table 1
(124)	cis-11-Eicosenoic	cis-Gondoic	$C_{20}H_{38}O_2$	Menhaden
(125)	trans-11-Eicosenoic	trans-Gondoic	$C_{20}H_{38}O_2$	Menhaden
(128)	cis-13-Docosenoic	cis-Erucic	$C_{22}H_{42}O_2$	Herring (305)
	Unsaturated - polyenoic			
(169)	11,14-Eicosadienoic		$C_{20}H_{36}O_2$	Herring, Menhaden

Fatty Acids Found in Fish Oils (Cont'd)

(170)	5,13-Docosadienoic		$C_{22}H_{40}O_2$	Fish oils
(172)	17,20-Hexacosadienoic		$C_{26}H_{48}O_2$	Fish oils
(175)	6,9,12-Hexadecatrienoic		$C_{16}H_{26}O_2$	Herring, Menhaden
(177)	7,10,13-Hexadecatrienoic		$C_{16}H_{26}O_2$	Menhaden
(180)**	cis-6,cis-9,cis-15-Octadecatrienoic	γ-Linolenic	$C_{18}H_{30}O_2$	see Table 1
(191)**	cis-9,cis-12,cis-15-Octadecatrienoic	α-Linolenic	$C_{18}H_{30}O_2$	see Table 1
(196)	5,8,11-Eicosatrienoic		$C_{20}H_{34}O_2$	Fish oils
(197)	8,11,14-Eicosatrienoic		$C_{20}H_{34}O_2$	Fish oils
(205)	6,9,12,15-Hexadecatetraenoic		$C_{16}H_{24}O_2$	Herring, Menhaden
(214)	6,10,14,18-Eicosatetraenoic		$C_{20}H_{32}O_2$	Fish oils
(222)	4,8,12,15,19-Docosapentaenoic	Clupadonic	$C_{22}H_{34}O_2$	Fish oils
(223)	7,10,13,16,19-Docosapentaenoic		$C_{22}H_{34}O_2$	Herring

* Extracted from K.S. Markley (Table 42) in Altman and Dittmer (049), except where otherwise stated.
** Linolenic acid is stated to have two isomeric forms (α and γ) with indistinguishable properties.

Table 3

Some Chemical Characteristics of Fatty Acids Found in Fish Oils*

FASEB Serial No.	Iodine No.**	Molecular weight	Melting point °C	Boiling point °C (at mm Hg)	Specific gravity (at °C)***	Refractive index**** (at °C)	Soluble in*****
(14)	0	228.4	53.9	250(100)	0.8622(54)	1.4273(70)	acet,alc,eth,pet.eth.
(16)	0	256.5	63.1	268(100)	0.8487(70)	1.4309(70)	acet,halc,eth,pet.eth.
(18)	0	284.5	69.6	213(5)	0.8390(80)	1.4337(70)	acet,halc,eth,pet.eth.
(20)	0	312.5	76.5-77.0	201(1)	0.8240(100)	1.4250(100)	bz,chl,eth,pet,eth.
(22)	0	340.6	81.5	306(60)	0.8221(100)	1.4270(100)	sl.sol:alc,eth.
(95)	99.77	254.40	-0.5 to +0.5	140-141(5)	-	-	-
(124)	81.74	310.50	23-24 50	267(15)	-	-	alc,me.alc.
(125)	81.74	310.50	52-53	-	-	-	-
(128)	74.97	338.56	34.7	281(30)	0.8352(70)	1.4444(70)	eth,me.alc.
(169)	164.55	308.49	-	-	-	-	acet,eth,pet.eth.
(170)	150.83	336.54	-	-	-	-	-
(172)	129.28	392.64	61	-	-	-	eth,pet.eth.
(175)	304.12	250.37	-	-	-	-	alc,eth.
(177)	302.12	250.37	-	-	-	-	acet,alc,eth,pent.
(180)	273.51	278.40	-11.3 to -11	125(0.05)	0.9164(20)	1.4800(20)	acet, me.alc,eth,pet,eth.

Table 3 - Cont'd.

(191)	273.51	278.40	-11.3 to -11	125(0.05)	0.9164(20)	1.4678(50)	acet,alc,eth,pet.eth.
(196)	248.48	306.45	-	-	-	-	CS_2,me.alc,hept.
(197)	248.48	306.45	-	-	-	-	CS_2,me.alc,hept.
(205)	407.98	248.45	-	-	-	1.4870(29)	CS_2,acet,alc,eth,pent.
(214)	333.48	304.44	-	-	0.9263(20)	1.4935(20)	acet,me.alc,eth,pet.eth.
(222)	384.0	302.49	-78	207-212(2)	0.9356(20)	1.5014(20)	acet,eth,pet.eth.
(223)	384.0	332.49	-	-	-	-	bz,chl,me.alc,pet.eth.

* Identified in Table 2, cf. FASEB serials Numbers
** Grams of I absorbed by 100 g of acid.
*** Referred to water at 4°C.
**** At the D-line of Na (589 nm).
***** sl = slightly; sol = soluble; acet = acetone; alc = alcohol (ethyl unless otherwise stated); h = hot; me = methyl; eth = diethyl ether; pet = petroleum; h = heptane; pent = pentane; bz = benzene; chl = chloroform. Gaps mean no data reported in Table 42 of Altman and Dittmer (049).

using two groups of 50 rats. They found that about 2% of each of these oils could be categorized as EFA using the dermal symptoms of EFA deficiency as the criterion.

Fish oils also contain volatile short-chain acids. Chipault and McMeans (176) collected 25 ml of volatile compounds during distillation of 5 gallons of menhaden oil. Paper chromatography indicated that these included formic (or acetic), acrylic, propionic, crotonic, butyric, and valeric acids; not all of the compounds were identified.

Despite their variability, fish oils as a class are distinguished by their high contents of fatty acids with chains longer than 18 carbons, and with four or more double bonds. Most fish oils are also rich in lipids other than FA and triglycerides: these include phospholipids, ether-linked compounds, hydrocarbons, wax esters, and sterols. In reviewing the compositions of fish oils Malins (558) and Stansby (836) remarked on the incompleteness of current knowledge. In general, lecithin and cephalin together account for about 75% of the phospholipids: most of the ether-linked compounds are found as diacyl glyceryl ethers; squalene is said to be the principal hydrocarbon· and sterols comprise mainly cholesterol and related substances including "vitamin" D. Table 4 illustrates the FA composition of lecithins in menhaden, salmon and tuna oil.

Lambertsen and Holman (501), partly characterized the hydrocarbons of herring oil by gas chromatography after various separation procedures. Among the straight-chain paraffins the odd carbon numbers were found to predominate, especially 27.0; among the branched-chain hydrocarbons, pristane (16.8) was 70%, and squalene (26.3) only 13% (Table 5).

Table 4

Specific Distribution of the Principal Fatty Acids from the Lecithins of Fish (622)

Fatty Acids	Weight Per Cent of Specific Fatty Acid Isolated From								
	Salmon			Menhaden			Tuna		
	Total	α'	β	Total	α'	β	Total	α'	β
14:0	0.7	1.9	0.6	0.5	Tr	1.3	0.7	Tr	1.0
14:1	—	—	—	Tr	Tr	Tr		Tr	1.8
16:0	22	37	10	45	78	6.2	19	36	5.0
16:1	8.0	1.4	3.8	Tr	0	Tr	2.1	Tr	2.3
18:0	2.0	2.7	Tr	3.2	5.9	0.9	5.0	7.9	1.0
18:1	6.7	8.2	23	7.4	2.3	13	7.6	6.4	16
18:2	Tr	Tr	Tr	1.0	Tr	2.4	0.7	Tr	2.2
18:3	Tr	Tr	Tr	Tr	Tr	Tr	0.8	Tr	1.1
20:4	1.0	0.6	0.3	2.2	Tr	0.9	2.7	1.3	5.0
20:5	12	14	17	13	1.7	29	7.1	8.4	15
22:1	0	0	0	0	0	0	0	0	0
22:4	Tr	Tr	Tr	Tr	Tr	Tr	Tr	0	0.5
22:5	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
22:6	43	33	46	28	12.5	42	55	39	48

From Menzel and Olcott (1961).

Table 5

Hydrocarbons Occurring in Herring Oil as Determined by Gas-Liquid Chromatography (501)

Straight-Chain Components		Branched-Chain Components	
Carbon Number	% Area	Carbon Number	% Area
14.0	0.3	15.4	3.5
15.0	7.5		
16.0	0.6	16.8 (pristane)	70.0
17.0	9.0		
17.6	2.2	17.9	5.0
18.0	1.7		
19.0	10.5	19.0	0.5
20.0	0.3		
21.0	6.0	19.9	1.0
22.0	0.3		
23.0	0.8	21[1]	3.5
24.0	0.3		
25.0	9.5	22.2	3.5
26.0	Trace		
27.0	33.0	26.3 (squalene)	13.0
28.0	Trace		
29.0	9.0		
30.0	Trace		
31.0	~7		
32.0	(Trace)		
33.0	~1		

[1] Flat and broad peak.

From Lambertsen and Holman (1963).

Kritchevsky et al. (496) studied the nonsaponifiable fraction of various sea foods for sterol content, and found that in three teleosts (haddock, pollock, and salmon) cholesterol accounted for more than 90%.

The effects of catalytic hydrogenation on these lipids have received relatively little study. Lambertsen et al. (503) took five samples of industrially hydrogenated marine oils, fractionated them by thin-layer chromatography, and analyzed the fractions by gas-liquid chromatography. "The general fatty acid composition was 30-45% saturated acids, 45-55% monoenoic acids, and 10-20% polyenoic acids. A substantial part of the polyenoic acids were trienoic acids, the rest dienoic. The presence of tetraenoic acids could be ascertained in the low-melting samples (mp 30-32°C)."

Ackman et al. (028) stated: "The partial hydrogenation of fats and oils to produce a stable, palatable product of suitable plasticity results in the formation of new, structurally altered fatty acids which in most cases differ chemically from familiar natural products.' The chemical aim of hydrogenation is selectively to saturate the polyene chains, but not the monoene.

Two other effects of hydrogenation have been reviewed by Hannewijk (324)--migration of double bonds, and cis-trans isomerization. These effects have been studied lately in monoene FA by Lambertsen et al. (502) and by Ackman et al. (028). No reports were found of studies on polyene FA.

Thus, both the original composition of fish oil and the effects of part-hydrogenation are variable and somewhat unpredictable. Therefore, the composition of Fish Oil (Hydrogenated) cannot be described within

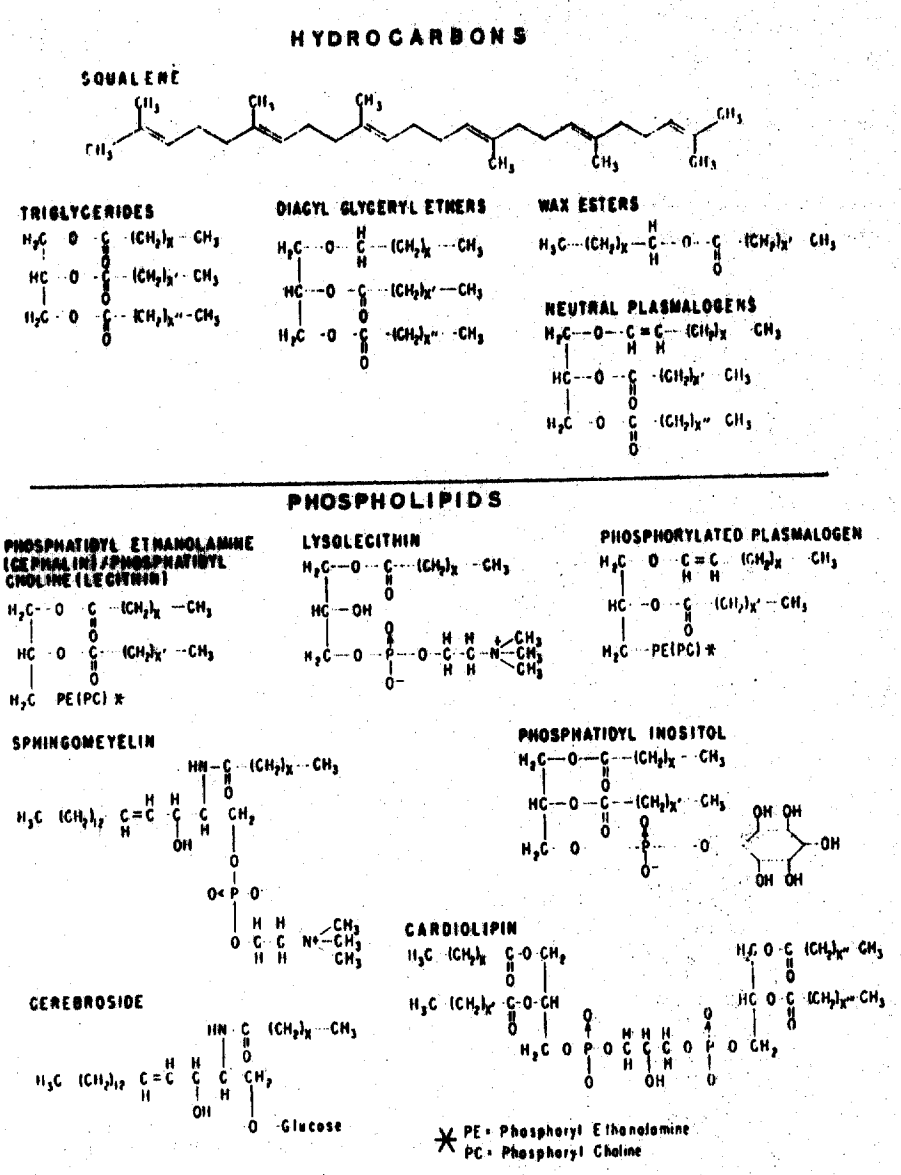


Figure 1. Structure of Some Common Lipids of Fish. From Malins (558).

narrow limits of components and their quantitative distribution. Large variations among samples can be expected. Nevertheless, the hydrogenation of fish oils does not make them resemble other hydrogenated oils. This was demonstrated long ago by an elegant series of cooling curves (900). Nor do hydrogenated oils from different fish species come to resemble each other chemically (864).

III. Structural formula

No single structure exists. Examples of the structures of some components of fish oils are given in Figure 1.

IV. Molecular weight

Data for the unhydrogenated FA components of fish oils are given in Table 3. Molecular weights of hydrogenated fish oils vary according to composition and degrees of hydrogenation.

V. Specifications

Fish Oil (Hydrogenated) is not mentioned in the Food Chemicals Codex, 2nd Edition, 1972 (188), the National Formulary, 13th Edition, 1970 (053), the U.S. Pharmacopoiea, 18th Edition, 1960 (898), or Synthetic Organic Chemicals, U.S. Production and Sales (U.S. Tariff Commission) (899). Nor are fish oils mentioned (unqualified by the word "hydrogenated").

According to Stansby (828), Codex Alimentarius standards have been recommended for fish oils. However, the current FAO Books in Print (267) contains no individual Codex Alimentarius standard for fish oils. Therefore, fish oil (hydrogenated) will be covered by FAO publication

No. CAC-RS 19-1969, General Standard for Edible Fats and Oils Not Covered by Individual Codex Standards (181).

Specifications relevant to the identity and toxicity of hydrogenated fish oils found in CAC-RS 19-1969 include the following:

3.5 Peroxide Value not more than 10 milliequivalents of peroxide oxygen/kg fat or oil

5.5 Copper (Cu): Non-virgin oil: 0.1 mg/kg

5.7 Arsenic (As): 0.1 mg/kg

7.1.2 Where an oil has been subject to any process of esterification or to processing which alters its fatty acid composition or its consistency the specific name of the oil shall not be used unless qualified to indicate the nature of the process.

VI. Description

Table 3 gives some physical properties of fatty acids of unhydrogenated fish oils; there are many gaps in the data. Table 6 similarly illustrates some properties of entire unhydrogenated fish and other oils. Hydrogenation alters these properties and also the flavor of fish oils. Figure 2, taken from Chang (172), summarizes the processing of fish oils.

Hannewijk (324) has reviewed the hydrogenation process. Oils are hardened to an over-all melting point (mp) of 35-37°C, monitored by sampling. A crude oil with more than 0.1% of FFA must be bleached and neutralized before hydrogenation. Other problems include high levels of bound aldehydes; however, most impurities are removed by normal refining. Added H_2 should be free from S and CO. If oil becomes hotter

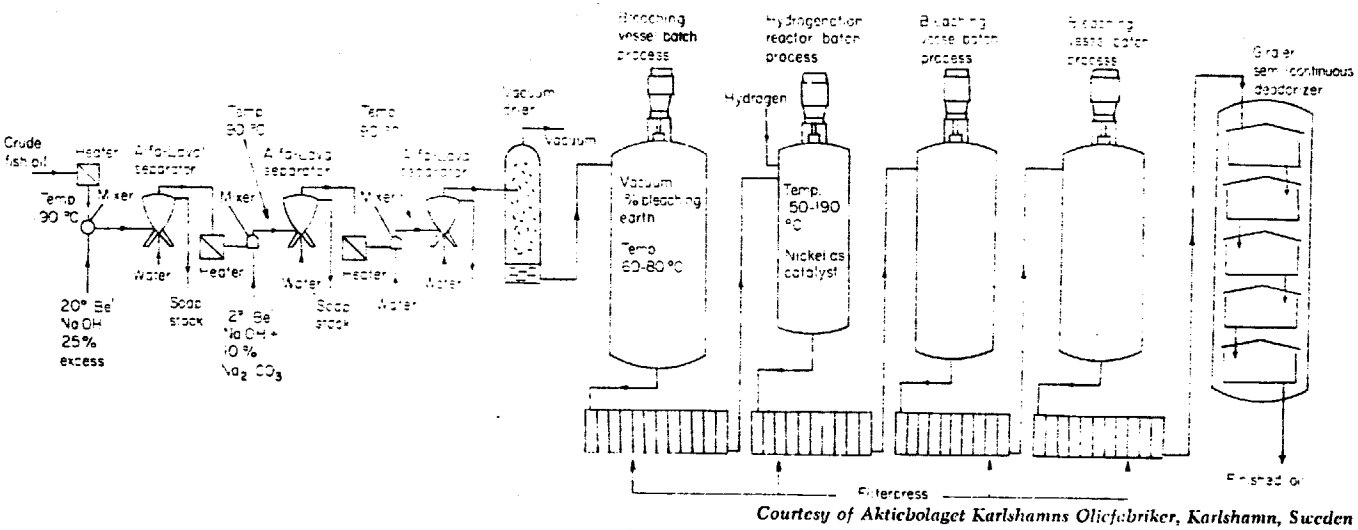
Table 6

Some Physical and Chemical Constants of Fish Oils and Other Oils

Type of oil	n_D^{65}=1.4* limits	n_D^{65}=1.4* mean	Iodine value limits	Iodine value mean	Slip point (°C) limits	Slip point (°C) mean	Saponification value limits	Saponification value mean
Stansby (833)								
Herring	573-602	565	123-148	134	9-17	14	181-187	184
Menhaden	590-623	608	150-165	159	22-28	24	192-199	196
Fineberg and Johansen (263)								
Herring			120-150				185-195	
Menhaden, Atlantic			150-185				185-190	
Menhaden Gulf			145-170				**	
Soybean			120-141				189-195	
Rapeseed			97-108				170-180	

* "A relationship exists between iodine value and refractive index such that I.V. = (n_D^{65} - 1.4429) x 8560." (833).

** Reliable data said to be unavailable (263).



Courtesy of Aktiebolaget Karlshamns Oljefabriker, Karlshamn, Sweden

Figure 2. Flow Sheet of Fish-Oil Processing. From Chang (172).

than about 200°C, aromatic FA (bound to glycerol) tend to be formed; however, high temperatures improve the selective hydrogenation of higher PUFA. A solid, preferably Ni-containing, catalyst of known pore size is used. Its contamination with S enhances cis-trans isomerization resulting in a fat that is firmer at room temperatures; however, S contamination accumulates, and must be watched. The kinetics of hydrogenation have been studied in detail by Miyake et al. (635). Koritala (491) has described Cu-containing catalysts, and Beal et al. (088) give a method for removing Cu residues from hydrogenated oils.

So the hardening of oils by hydrogenation results from two sorts of chemical alterations--saturation of double bonds, and cis-trans isomerization. The relative amounts of these alterations and their control, are the subject of patents by which manufacturers seek to guard the individuality of their branded products (621,624.625,649). So, also, hydrogenated oils and fats are mixtures of lipids with very different mp and expansion spectra, and the latter are described as "steep" or "flat".

The hydrogenated fats are treated with weak alkali to remove traces of FFA and the catalyst, and are then bleached and deodorized. According to Hannewijk the finished oils or fats should be stable for about three months, which is longer than the usual shelf-life of margarine in Western Europe (for American practice, see Consumer Exposure). If the oils are processed carefully, antioxidants are not needed; if not, they are of little avail. This was also the opinion of Lea (512).

"Hardening flavors" resulting from hydrogenation are attributed to the presence of free ketones and especially free aldehydes, for which

taste thresholds can be very low, e.g., 0.0003 ppm for 6-*trans*-nonenal. Much higher levels of some aldehydes can develop within hours in daylight and within about two months in the dark. Hardening flavor also varies with the mp spectrum and the degree to which PUFA are saturated. Furthermore, human taste thresholds vary enormously, see review by Carr *et al.* (164). Hannewijk states that hardening flavors can almost always be perceived in herring oils of mp 30°C or less, are "largely removed" at mp 33°C, but are occasionally present even at mp 35°C. Menhaden oil, however, must be hardened to mp 40°C or more to eliminate hardening flavors.

All authors emphasize that most of the keeping qualities of hydrogenated fish oils depend on careful refinement *before* hydrogenation.

VII. Analytical methods

The CAC General Standard for Edible Fats and Oils (181) lists acceptable methods for determining acid value; peroxide value; matter volatile at 105°C; insoluble impurities; soap content; and contents of iron, copper, lead, and arsenic. No specific CAC Standard has yet been issued for fish oils or hydrogenated fish oils.

Malins and Mangold (560) and Mangold (564) described the analysis of complex lipid mixtures by methods centering on thin-layer chromatography (TLC). In a later paper, Malins and Wekell (561) described the classification of lipids by adsorption chromatography, using columns or TLC and a variety of adsorbents. Many other fractionation procedures are used for determining useful components of fish oils; for example, Pohle *et al.* (718) reported measurement of solids by nuclear magnetic resonance.

Houle (380) has determined the FA composition of fish oils by gas-liquid chromatography. Ackman et al. (028) used open tubular gas-liquid chromatography to analyze a hydrogenated herring oil of mp 32.5°C, iodine number 76, 30% saturated FA, and 66% monounsaturated FA. They found that they could determine the cis and trans isomers.

Stansby (835) reviewed procedures for analyzing complete fish oils for saturation (iodine number), FFA, moisture, color, and impurities, by standard procedures listed by the Association of Official Agricultural Chemists (070) and in the Official and Tentative Methods of the American Oil Chemists Society (052).

Flavors, however, cannot be determined adequately without using a human sensory evaluation panel. Such a panel cannot render determinations or exact measurements (164).

Banks (082) reviewed deteriorative changes in fish oils, including the slow formation of FFA by hydrolysis in the presence of alkali or of lipases (enhancing autoxidation), and the more important autoxidation that can lead to formation of hydroperoxides and thence epoxides (see Section B. II). The standard test for determining oxidation products of fish oils at present is the 2-thiobarbituric acid test (222).

Impurities arising from the environment or from processing of fish oils include copper, arsenic, and bromine. Analyses more recent than, or not included in, the CAC General Standard (181), that have been found in the literature, are as follows:

Deck and Kaiser (225) developed a colorimetric analysis of edible oils for copper content. Lunde (541) described a neutron activation

analysis for arsenic and bromine after fractionation of oils on silica-gel columns.

All of the above methods are described for fish oils in general. The following methods have been described for hydrogenated fish oils:

Ueno (901) determined thiocyanogen value as a measure of the degree of hydrogenation. Sardine oil showed a rapid decrease of thiocyanogen value at the stage of hydrogenation where the iodine number fell to about 70-80.

Menzel and Olcott (622) described an enzymatic analysis for positional distribution of FA in the phosphatidyl choline (lecithin) fractions from various animals and fish including tuna and menhaden.

Lambertsen et al. (602) lately developed a fractionation procedure and gas-liquid chromatographic analysis for monoene FA isomers in hydrogenated fish oils.

VIII. Occurrence and Levels Found in Plants and Animals

A. Plants. Not applicable. However, it is well known that some, if not all, fatty acids can move up the food chain unaltered.

B. Animals. See Sections II and VI above.

C. Synthetics. Not applicable.

D. Natural Inorganic Sources. Not applicable.

BIOLOGICAL DATA

I. Acute Toxicity

Matsuo studied the toxicity of fish oils for many years. His first reported finding was that rats fed diets containing 5% of ethyl esters of highly unsaturated FA from cuttlefish oil thrived, but the oxidized forms of these esters were toxic (586). The degree of toxicity was found to be directly proportional to the peroxide content which could result from autoxidation of the oil (585). "Profound damage" to the mucous membranes of stomach and intestines of rabbits, and liver damage, resulted from oral administration of 0.8 ml per day of the autoxidized esters, and topical application of these or non-oxidized esters to the back skin of rats had like effects (598). Rats were similarly affected by peroxides formed in cod liver oil by autoxidation (597). After various experiments involving heat-polymerization of cuttlefish oil and of ethyl linolenate, both of which gave rise to cyclic esters that proved to be acutely toxic to rats, Matsuo purified a cyclic monomer of mw 282. This proved rapidly fatal to rats when fed as 20% of a basal diet, and Matsuo concluded that this monomer was the toxic endproduct of autoxidation and peroxidation of fish and other oils (599).

II. Short Term Studies

Reports of fish oil toxicity in animals center on products of oxidation and deficiencies of vitamin E. These can end in damage to growth mechanisms, muscular dystrophy, multiple sclerosis, and brain damage. In these cases, however, the material fed would probably not have satisfied any regular quality standards for edible hydrogenated

fish oils, and the reports do not discuss extrapolation of the data to man. No reports of chronic fish oil toxicity in man have been found.

Dam (202) studied the production of exudative diathesis and of encephalomalacia as symptoms of vitamin E deficiency in chicks. He found that both symptoms were accelerated by feeding highly unsaturated long-chain FA; the source of such FA determined the relative prominence of the symptoms, and so did the protein/carbohydrate ratio of the diet. Addition of 1% of cholesterol accelerated the appearance of exudates when the diet contained 5% cod liver oil, and counteracted encephalomalacia when the diet contained 30% lard. Dam concluded that tissue damage, and not merely loss of antioxidant properties in the diet, caused the symptoms. He failed to reproduce either of the symptoms in rats, rabbits or ducklings; however, rabbits developed muscular dystrophy when fed a diet deficient in vitamin E.

Gershoff and Norkin (286) fed purified diets with various levels of vitamin E, with or without 5% tuna oil, to immature cats for up to 13.5 months. Cats fed tuna oil and no vitamin E developed steatitis with low levels of linoleic acid in their adipose tissue. Vitamin E supplements protected the cats completely against steatitis. When neither vitamin E nor tuna oil was added to the diet, mild symptoms of vitamin E deficiency, including muscle changes, developed after 12 months. These findings were interpreted in terms of the dietary requirements of the cat.

Harris and Embree (333) studied the relationship between intakes of PUFA and requirements for vitamin E in man. They calculated the average intakes of PUFA in grams per head for the population of the

United States for the year 1960; the average intakes of vitamin E were expressed as mg of d-α-tocopherol. The ratio, mg of vitamin E to g of PUFA, was found to be 0.6, and the authors inferred from published data that this ratio was probably marginal for protection of man against vitamin E deficiency. Fish oils were minimally involved in this study. Very recently the National Academy of Sciences has issued a denial of suggestions that vitamin E deficiency may be widespread in the United States at the present time (657).

Njaa et al. (668) included hydrogenated marine fat (HMF) (mainly from herring oil, unhydrogenated herring oil, or other fats) as 50% of the calories in a diet containing starch, sucrose, and albumin (10% w/w). These diets were fed to 29 groups of month-old rats for four weeks. Rats fed HMF grew relatively slowly and developed enlarged livers not due to fat, while rats fed the unhydrogenated herring oil grew better and developed fatty livers. The authors attributed the results of HMF to the presence of 20- and 22-carbon FA "of low saturation" together with trans and positional isomerization of the FA, in the HMF as fed.

Blaxter et al. (113) fed recently born bull calves a milk diet plus amounts of cod liver oil up to 4 oz per day for up to 71 days. Some calves died during the experiment; others were killed at the end. Autopsies revealed severe dystrophy of skeletal and heart muscles, but no lesions in livers, kidneys, or nervous systems. Partial protection was given by supplements of vitamins A and D.

Blaxter et al. (111) confirmed and amplified these findings "clinically, chemically, on dissection and histologically." They showed that the cause was the total unsaturated FA fraction of the

cod liver oil. No dystrophy was caused by the saturated FA fraction, the less unsaturated portion of the PUFA fraction, or the unsaponifiable residues of the cod liver oil. Only slight dystrophy was caused by the more unsaturated portion of the PUFA fraction. The tocopherol content of muscle samples did not differ as between normal and dystrophic muscles. No peroxidation of body fats was found. The authors concluded that the dystrophy was a general effect of the total PUFA fraction of the cod liver oil, and was unconnected with any hypervitaminosis A or D.

Subsequently Blaxter et al. (112) found that DL-α-tocopheryl acetate, given orally but not when given parenterally, protected similar calves against muscular dystrophy from cod liver oil supplements. Daily administration of 1 g of methylene blue gave similar protection and did not affect tissue levels of tocopherols. Ascorbic acid, ethyl gallate, or biotin gave no protection. The authors came to no conclusions about the mechanisms of these effects, but suggested a connection with oxidative phosphorylation.

A different picture emerged from four experiments on rats reported by Ershoff (251). Diarrhea and retarded growth resulted from supplementation of a low-fat, purified diet with crude or refined oils of tuna, sardine, menhaden, or cod liver. Substantial protection was given by additional supplements of cottonseed, soybean, sesame, corn, or wheat germ oils, by α-tocopherol, DPPD (N,N'-diphenyl-p-phenylenediamine), or Santoquin (6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline), or by alfalfa meal, dessicated liver N.F., Torula yeast, casein, fish meal, DL-methionine, or a mixture of amino acids.

No protection was given by olive, coconut, or hydrogenated cottonseed oils; by butter fat or lard; by methyl linoleate; or by α-tocopheryl acetate or phosphate. The author drew no conclusions except in the introduction to his paper, where he remarked that "toxic effects may occur following fish oil administration due to factors other than the induction of a vitamin E-deficiency state."

Many investigators, in addition to Matsuo (see Acute Toxicity), have shown that toxic effects of oxidized oils can be attributed to peroxides and not to carbonyl derivatives.

Andrews et al. (058) studied the toxicity to rats of peroxides formed by autoxidation of soybean oil. They found that the peroxides were destroyed in the intestine, and concluded from this and other indications that the intestine was the site of toxic action. Inhibition of intestinal xanthine oxidase activity by autoxidized soybean oil and its reversal by exogenous FAD cofactor suggested interference with intestinal enzyme activities as a possible mechanism of peroxide toxicity. In these experiments this toxicity was demonstrated when the peroxide value of the added oil was as low as 100.

Rasheed et al. (739) fed rats a diet supplemented with 10% of pure triglycerides from fresh menhaden oil, and all was well; but when the triglycerides were oxidized experimentally to peroxide values of 125-310 they caused steatitis, enlarged livers, and high malonaldehyde (carbonyl) levels in blood and excreta. When the fresh oil was allowed to autoxidize for 24 hours and then was fed as 15% of the diet, it produced anorexia, steatitis, low hemoglobin,

and death, depending on the degree of autoxidation. Symptoms were largely reversed when rats, fed oil autoxidized to a peroxide value of 60, were treated with ethoxyquin or α-tocopherol; the rats were protected when these antioxidants were added to the fresh oil, but α-tocopheryl acetate, for example, was less effective than DL-α-tocopherol. High carbonyl levels in the blood were considered to be "a useful diagnostic criterion" for toxic effects of peroxidized oils.

However, Carpenter et al. (163) found no depression of growth in chicks fed de-fatted herring meal supplemented with herring oil pre-oxidized to a peroxide value of 142.

Roubal and Tappel (760) studied the effects of peroxidizing lipids, typified by ethyl arachidonate, upon the integrity of proteins in vitro. They found that transient free radicals, generated in the reaction mixture, damaged 17 different amino acids in proteins that included gamma-globulin, catalase, bovine serum albumin, ovalbumin, and hemoglobin. In addition, trypsin, pepsin, ovalbumin, α-chymotrypsin, cytochrome-c, and hemoglobin were rendered at least partly insoluble, and cysteine was partly converted to cystine with liberation of H_2S. This damage, according to the authors, "appears to be about one-tenth as effective as radiation damage."

Dam (204) illustrated the pathway by which peroxidic free radicals are generated during autoxidation of linoleic acid.

Reviewing these effects of autoxidation of fish oils, Stansby (832) emphasized that "These are all only potential effects. Whether or not they develop depends to a very large extent upon circumstances.... The most important factor influencing the degree to which these potential

alterations may develop is the degree and extent to which the polyunsaturates oxidize. This in turn is largely controlled by the use to which the oil is put." These remarks were directed to all fish oils, not especially to hydrogenated fish oils, in which the degree of polyunsaturation is, of course, diminished.

There appears to be a theoretical possibility that epoxides may arise either during metabolism of peroxides in the intestine or by action of drug-metabolizing enzyme systems on fish-oil hydrocarbons, and a further possibility that such epoxides may be carcinogenic. Grover (303) has reviewed the mechanisms. Apparently epoxides that form at the K regions (regions of high electron density associated with isolated phenanthrenoid double bonds) or other regions of cyclic hydrocarbons, are electrophilic. When these epoxides react with nucleic acids in vitro or in cultured cells, alkylation may occur; alternatively, an epoxide may be intercalated into DNA or may delete a DNA base, and either case will result in a frameshift mutation. Resultant morphologically abnormal cell colonies apparently have been found to be malignant on reinjection into rodents.

No reports have been found in which these effects have been studied in relation to hydrogenated fish oils in vitro or in vivo.

On the other hand, the rate of growth of carcinosarcoma in rats was reported by Haven (345) as slower when the dietary fat was cod liver oil of iodine number 9, than when it was coconut oil of iodine number 157. The difference was attributed to the difference in saturation of the FA.

III. Long Term Studies

No long term studies of chronic toxicity of hydrogenated fish oils have been found in the literature.

(Questions about hydrogenated PUFA, cholesterol deposition, and atherogenesis are outside the scope of this monograph. The present consumption of hydrogenated fish oils in the United States is virtually nil. Furthermore, the roles of saturated FA and blood cholesterol level in atherogenesis are matters of controversy, and cannot be debated here.)

IV. Special Studies

Pesticide residues. Pesticides of current concern that appear in sea fish comprise mainly the DDT family (DDT, DDE, and TDE). Over 5 ppm in fish or fractions of fish is unacceptable. Other substances that cause concern because of accidental contamination are the polychlorinated biphenyls (PCB). DDT and PCB are both toxic and persistent because they enter the food chain and stay there.

In 1968 Stout (848) monitored levels of the DDT family in several species of food fish in the Northwest Pacific and found levels up to 0.4 ppm; she attributed these accumulations to agricultural run-off by way of the Columbia River. In 1970 Stout et al. (849) reviewed these and other findings; for example, fish taken off Southern California had contained 20-50 ppm rising to 1,026 ppm, mainly in fat-rich tissues. Experiments had been undertaken to find ways of eliminating these residues; otherwise it would be useless to go fishing. It was found that all detectable amounts of p,p'-DDT in fish oils could be removed by refining, and 90% could be removed by hydrogenation; thus the regular processes (Figure 2) would normally eliminate all detectable

p,p'-DDT. However, p,p'-DDE, although substantially reduced, was not eliminated by these procedures. Stout et al. (849) concluded that the deodorization step was critical because temperatures were kept low (190-200°C) to avoid thermal degradation of the oil; therefore this step should be prolonged when pesticides of the DDT family were present or suspected.

These studies substantially confirmed earlier work by Smith et al. (816) on the removal of DDT, DDE, endrin, aldrin, dieldrin, heptachlor and heptachlor expoxide from vegetable oils.

Less work has been reported on PCB in fish oils. Beezhold and Stout (094) have described a gas chromatographic method of detecting PCB in fishery products. Problems have been encountered with standards, and studies are in progress. No reports have been found on the elimination of PCB from fish oils.

BIOCHEMICAL ASPECTS

I. Breakdown

Only oxidative changes seem to have been reported. Thermal degradation is not considered because hydrogenated fish oils have not been reported as used for deep-frying.

Possible toxic effects of oxidation have been discussed in foregoing sections. In addition, four sorts of flavor changes have been reported (828): (1) rancidity, (2) reversion to the "fishy" flavor of the original oil, (3) a "hardening" flavor resulting from hydrogenation and removed afterwards (see Figure 2), and (4) reversion to the hardening flavor.

These changes reportedly affect consumer acceptance, and much of the chemistry is obscure. The Codex Alimentarius recommendations (181) aim to define a product that is free from such defects. No reports have been found of tests using hydrogenated fish oils that specifically comply with those recommendations. Currently there is no significant use of hydrogenated fish oils in the food supply of the United States (see Consumer Exposure). The following data are mentioned because they may become relevant in the future.

Chipault (174) isolated volatiles from oxidized fish oils under vacuum at 100°C, by vacuum-steam distillation, and by adsorption from nitrogen gas onto charcoal. Paper chromatography of the acidic fractions revealed only acetic and propionic acids. The basic fractions contained small amounts of amines that were not identified. The carbonyl fractions contained formaldehyde, acetaldehyde,

propionaldehyde, butyraldehyde, valeraldehyde, hexaldehyde, acetone, butanone, pentanone, α-β-unsaturated monocarbonyls, glyoxal, other low-mw α-keto aldehydes and diketones, and 2,4-dinitrophenylhydrazones not further identified.

Wyatt and Day (948) isolated and measured quantitatively 26 carbonyl compounds from salmon oil autoxidizing under controlled conditions.

Meijboom (620) tried to relate the odors and tastes of some pure saturated and unsaturated aldehydes to their molecular structures. He found, as have others, that the technical problems were enormous; to date they remain unsolved (164). Nevertheless, he and his colleagues (458) appear to have identified the principal carrier of hardening flavor in hydrogenated linseed and soybean oils as 6-trans-nonenal, which was detected by some subjects at the concentration of only 0.3 ppb.

Hannewijk (324) has argued that if Keppler et al. are correct, 6-trans-nonenal should also carry the hardening flavor in hydrogenated fish oils. He has attributed the reversion of hardening flavor in hydrogenated fish oils to slight increases of bound aldehydes resulting from oxidation during storage.

According to Stansby (828), the stability and flavor of hydrogenated fish oils used in foods until 1951 presented problems; however, use of menhaden oil should cause fewer problems because it is less highly unsaturated. Menhaden oil is now, and foreseeably, the chief source of hydrogenated fish oils in the United States (see Consumer Exposure). Stansby adds that flavor reversion problems have been overcome with hydrogenated soybean oils, that fish oils contain similar ω-3 PUFA,

and that hydrogenated herring oils are used in the European food supply.

II. Absorption-Distribution

No indications were found in the literature that pathways of absorption and distribution of hydrogenated fish oils might differ from those of other dietary fats. In principle, ingested fats are digested to monoglycerides and FFA by bile action in the intestine; short-chain FA are absorbed into the bloodstream; and FA with chains longer than 8-12 carbons enter the lymph, diffuse into the thoracic duct, and filter gradually into the circulation. Hydrogenated fish oils are especially rich in long-chain FA (see Table 2).

Fats that are excreted in the feces normally have not been absorbed. Thus McCay and Paul (607) found that guinea pigs utilized about 60% of a hydrogenated vegetable oil, compared with 90% of unhydrogenated oils including salmon oil.

Fish oils polymerized by hydrogenation were reported by Matsuo (585,586,599) to damage the intestinal mucosa when fed to rats as sole sources of dietary lipids.

Beare (090) fed rats a variety of oils including partly hydrogenated herring oil and measured the FA distribution in liver, carcass, and milk fats. She found that the quantities of palmitic, palmitoleic, stearic, oleic, linoleic, linolenic, eicosenoic, eicosadienoic, and erucic acids in milk reflected the dietary pattern of FA, generally more than did the carcass FA. Tissue arachidonic acid, not provided in the diet, was related to dietary linoleic acid level. Changes of diet fat were followed by corresponding changes in tissue distribution

of long chain FA. Beare observed that the rats took a long time to adapt to utilization of erucic acid.

It is well known that some fatty acids, that are first synthesized in plankton, can persist through the food chain.

Mohrhauer and Holman (636) fed 108 weanling rats a fat-free diet plus purified linoleic and linolenic acids in several proportions and they determined the FA composition of lipids in heart, liver and adipose tissue by gas chromatography. Tissue levels of arachidonate were inversely related to amounts of dietary linolenate, showing that the linolenate inhibited conversion of linoleate to arachidonate. In another study, the same authors (637) fed 184 weanling rats ethyl linoleate, ethyl arachidonate, or ethyl linolenate, and measured the FA composition of the liver. It was found to correspond well with the diet.

Neudorffer and Lea (660) fed turkeys a basal diet plus 2.5% of beef fat or anchovy oil, or 5% of anchovy oil. The basal diet contained 2.3% of lipid richer in linoleate than either of the supplements. Analysis of skin fat revealed that FA had been transferred from the diet with high, though varying, efficiency. Peroxide values of the skin fat after incubation showed that the unhydrogenated anchovy oil had greatly reduced the stability of the skin fat.

Imaichi et al. (388) used gas-liquid chromatography to study the FA composition of 210 samples of depot fat removed at surgery from the abdominal or thoracic walls of human patients. Ten of these patients were known to be unusually high consumers of fish oils, and their

depot fat contained approximately double the levels of highly unsaturated FA found in the average of the other samples.

III. Metabolism and Excretion

The normal metabolism of FA present in fish oils has been studied; for example, "families" of FA tend to repress the metabolism of other FA "families". Thus, Holman and his colleagues (637) (372) found that increases of the linolenate/linoleate ratio in the diet progressively inhibited conversion of linoleate to arachidonate. Brenner and Peluffo (128) and Mohrhauer et al. (638) have extended Holman's findings. However, in these studies highly refined oils derived from fish oils were fed to animals as the sole dietary lipids, and the data probably do not illustrate what happens when fish oils contribute in small part to normal human diets. Nor did these studies involve partly hydrogenated fish oils of uncertain FA composition.

Hartwell (338) digested a number of different fats with pancreatic lipase in vitro. She found that coconut oil was the most rapidly digested; followed by palm kernel oil and castor oil; then butter fat; and lastly a slow group including beef and hog fats, a number of vegetable oils, cod liver oil, and hydrogenated whale and arachis oils.

The term "essential fatty acids" (EFA) originally meant FA that alleviated certain skin symptoms and growth failures in laboratory animals fed experimental diets. Later, EFA were identified as PUFA with the first double bond in the ω-6 position, e.g., linoleic acid (836). Then Privett et al. (728) (730) showed that PUFA of the linolenate series, having the first double bond in the ω-3 position, possessed

EFA activity when fed as 10% or more of experimental diets; they also found that fish oils were not inferior to corn oil as sources of EFA activity. However, these fish oils were not hydrogenated.

The EFA activity of partly hydrogenated fish oils has been studied by Aaes-Jørgensen since 1954 and also by Beare (090). The findings have been summarized by Aaes-Jørgensen (007) as follows:

> "However, only very few experiments have been made so far with partially hydrogenated fish oils as dietary fat.
>
> "In a study on the effect of partially hydrogenated marine oils as the sole dietary fat to rats, hydrogenated oil was used in an amount of 7% and 28% of an otherwise fat-free diet to weanling rats for 14 weeks, Aaes-Jørgensen (1954). The higher percentage of hydrogenated herring oil (28%) decreased the growth rate significantly as compared with the 7% level. The growth rate of the latter animals was significantly poorer than that of animals on 7% or 28% non-hydrogenated herring oil. Further, on the diet with 28% partially hydrogenated herring oil the animals ceased gaining weight after six weeks on the diet and began to lose weight after nine weeks. After 14 weeks on this diet the rats were moribund. The dermal symptoms indicating EFA deficiency were very pronounced in all the animals reared on diets with 7% or 28% partially hydrogenated herring oil. Hematuria developed in 50% of the animals fed on 7%, and in 100% of the animals fed on 28% hydrogenated herring oil. At the time when these experiments were carried out, the alkali isomerization procedure was the only available technique for determination of the unsaturated fatty acids. Thus, rather limited information only could be obtained concerning the fatty acids in the dietary fats. However, it was beyond doubt that the contents of polyenoic acid, normal as well as conjugated, were low.
>
> "Beare (1961) fed diets with 2% partially hydrogenated herring oil to male, weanling Wistar rats for six weeks. Gas chromatographic analysis of the oil indicated a low content of linoleic acid, the presence of odd-numbered fatty acids, and of several series of C_{20} acids. The rats fed on the hydrogenated herring oil consumed more food and gained more weight than did the animals fed on diets with similar amounts of corn oil, rapeseed oil, or margarine (possessing some marine oil). However, when all weight gains were adjusted for food consumption by covariance analysis, the diet containing corn oil appeared to be the most efficiently utilized, and the diet containing margarine the least; rapeseed and hydrogenated herring oil were intermediate.
>
> "The livers of the rats fed partially hydrogenated herring oil were significantly heavier than those of the other rats. Despite the small quantity of dietary linoleic acid available to these rats, the level of octadecadienoic acid in liver and carcass fat was similar to

that of rats fed on rapeseed oil or margarine. The author suggests a preferential retention of the sparsely available linoleic acid. In some livers there appeared to be an isomer of the common linoleic acid. The weight of the 14-15 days old litters of mothers fed on hydrogenated herring oil was significantly smaller than those of mothers supplied with corn oil or margarine. The rat milk fatty acids generally reflected more closely the dietary pattern of fatty acids than did those of the tissues.

"The nutritive effects of partially hydrogenated fish oils are only vaguely known and present a most difficult area to explore because of the complexity of the unsaturated fatty acid pattern of such oils. However, further studies in this area are needed and no doubt will be undertaken as the analytical techniques become more refined."

Aaes-Jørgensen and Hølmer (008) fed partly hydrogenated herring oil to rats as the sole dietary fat. Spermatogenic tissue was totally impaired after 5 weeks, contrasted with 15 weeks on partly hydrogenated arachis oil, and 26 weeks on fat-free diet. These findings were attributed to EFA deficiency.

Other reports attribute losses of nutritive value in fish oils to autoxidation. For example, feed intake, weight gain, and hemopoiesis diminished with increasing oxidation of menhaden oil fed to rats (739) and pigs (681). Stansby (836) has interpreted these data and others already noted (202) (113) (111) (112) (286) in terms of EFA deficiency.

As already noted (see Description), careful refinement should not destroy antioxidant properties of the original fish oils; however, more can be added (181). Dam (204) reviewed the activities of a range of antioxidants. Harris and Embree (333) estimated that 0.6 mg of D-α-tocopherol per g of polyenoic acids should supply the required amount of antioxidant activity. However, Olcott (676) has pointed out that the antioxidant activity of α-tocopherol is not commensurate with its total vitamin E activity.

The impairment of vitamin activities in fish oils by part-hydro-

genation does not seem to have been studied quantitatively. Dubin and Funk (235) reported that part-hydrogenation of cod liver oil in the presence of Ni at temperatures not over 115°C did not destroy vitamin A and D activities. However, van der Steur (913) found destruction at the higher temperatures of modern refining processes, though vitamin E survived better than A or D. He pointed to risks of over-supplementation of finished oils with synthetic vitamins A, D, or E.

Paluszak (693) hydrogenated a vegetable oil to mp 59°C and iodine number 5, eliminating linolenate, linoleate, and arachidonate. He fed this oil as a diet supplement to groups of young rats, with and without added cholesterol and biotin (1% and 2 ppm respectively in the diet). Losses of weight and of depot fat during 11 weeks of feeding were attributed to practical deficiencies of biotin and of EFA associated with the extreme hydrogenation.

Nevertheless authors mostly agree that partly hydrogenated fish oils, properly prepared, are nutritionally adequate sources of dietary fat (902) (913) (836).

As already noted, fecal excretion of significant amounts of dietary fats indicates failure of absorption (607). It is common knowledge that a major route of excretion of fats is through sweat. However, most fats are utilized by the body as energy sources.

IV. Effects on Enzymes and Other Biochemical Parameters

Bernsohn and Stephanides (100) have argued that multiple sclerosis can be caused by PUFA deficiency that results in demyelination of the nervous system. Kishimoto et al. (468) analyzed slices of the left

cerebral hemispheres of patients who died of multiple sclerosis and found subnormal axon densities and ganglioside concentrations in the plaques, and also a fatty acid distribution in the glycerophospholipids of the plaques that resembled grey matter rather than adjacent white matter. The white matter resembled that from control slices. It should be noted that hydrogenated fish oils do not figure in these reports.

The extensive literature on cholesterol-lowering effects of fish oils has been reviewed by Stansby (836). Although various explanations have been proposed for the "superior cholesterol depressant effect of fish oil fatty acids," the mechanisms remain "obscure." All of these explanations concern FA of fish oil before hydrogenation; the relevance to hydrogenated fish oils is not discussed. Stansby (836) also regards as speculative reports that glyceryl ethers and non-FA components of fish oils, such as squalene, have desirable nutritional properties.

Grollman (301) and Reichsman (746) reported that fish oils have properties that can be used to lower blood pressure in patients with chronic hypertension, but which do not alter blood pressure in normotensives. These reports did not involve hydrogenated oils.

No reports have been found of any use of hydrogenated fish oils to study the well known inhibitory effects of FA on the activities of some carbohydrate-metabolizing enzymes.

V. Drug Interaction

No reports have been found of studies of drug interaction. Neither have reports been found to suggest that hydrogenated fish oils differ

from other lipids in capacity to carry fat-soluble vitamins A, D, E and K.

VI. Consumer Exposure Information

Statistics for 1971 (Table 7) disclose heavy United States trade in fish oils but "negligible" use of hydrogenated fish oils in foods. Reasons for this have been discussed by Stansby and Knobl (839) and Stansby (828). During World War I, Californian sardines (pilchards) became important as a food source, and state law restricted their uses to food. By 1920 hydrogenated fish oils were used in margarine in Europe, but laws restricted the production and sale of all margarine through much of the United States. In 1925, 120,000 lbs of hydrogenated sardine oil were used in margarine in the United States; this figure rose to 40 million lbs in 1940, when less than half of these amounts of menhaden oil were so used. Additional unrecorded amounts of hydrogenated fish oils were used in shortenings. By 1951, however, overfishing had destroyed the California sardine stock and no fish oils were made into margarine or shortening in the United States from that year until 1967, when some herring oil was imported from Iceland for use in shortenings. This practice was quickly discontinued for fear that labeling requirements would force its disclosure.

The present FDA standard of identity for oleomargarine (067) does not permit the use of fish oils. When this standard was enacted in 1955 no petitions were received for the inclusion of such fish oils, and they were therefore omitted by default. No evidence for or against the use of fish oils has been considered by the FDA--either up to that

Table 7

Fish Oils - Consumer Exposure Tables

Production*	1971	1970	1949
World production, millions tons:			
Marine, vegetable, animal oils	41.373	39.236	
Vegetable oils	20.505	19.545	
Marine oils	1.261	1.292	
Fish oils including liver oils	1.095	1.052	
USA total of fats and oils, millions lbs:			
Food use	11,009	11,161	6,419
Non-food use	5,383	5,675	3,735
USA production of shortenings,** millions lbs:	3,479	3,599	1,494

Imports into USA, millions lbs***	1971	1969	1965
Cod oil except liver oil	5.974	3.764	4.765
Herring oil except liver oil	1.470	0.055	0.771
Other fish oils, NSPF, except liver oils	0.076	0.124	0.002
Sperm whale oil**** crude	41.437		
refined	4.115		

* These data were abstracted from Agricultural Statistics, 1972, US Government Printing Office, Washington, D.C. (897)

** Content of fish oils called "negligible"

*** These data were abstracted from US Imports for Consumption and General Imports, US Department of Commerce, Bureau of the Census, publications FT 246-71, FT 246-69, and FT 246-65, US Government Printing Office, Washington, D.C. (896)

**** Imports now (1973) illegal.

time or since. However, the recent internationally recommended Codex Alimentarius specifications for margarine (182) permit the use of fish oils; the present U.S. standard of identity for oleo-margarine (067) is about to be reconsidered by the FDA (066). If no petition is made to include the use of fish oils, the present law will continue to require their omission from the standard of identity. On the other hand, if a petition is presented, evidence will be called for, and the Select Committee on GRAS substances review of hydrogenated fish oils will be potentially relevant evidence.

No such legal restructions apply to the use of hydrogenated fish oils in shortenings in the United States. However, any plant in which a food component is prepared must meet sanitation requirements that have been adopted by the FDA; according to Stansby (828) "few, if any, fish oil plants in this country meet such requirements". This stricture does not apply, of course, to fish oils imported into the U.S. from countries where plants have met the FDA sanitation requirements, for example, the herring oil that was imported from Iceland in 1967 (828).

Practical inhibitions on resumption of the use of fish oils for food purposes in the United States include problems of price, public image, and technology concerned with elimination of undesirable trace components and flavors (828). The elimination problems appear to be soluble (828). Public images are notoriously malleable, and the 1973 increases in the price of soybean oil are relevant to any future demand for reintroduction of fish oils. Some data on world trade in fish oils 1962-1967 are given by Stansby and Knobl (839).

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